BCG Vaccination and GAD65 and IA-2 Autoantibodies in Autoimmune Diabetes in Southern India

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ABSTRACT: **This paper reports a study to determine whether BCG vaccination is associated with an increase or decrease in GAD65 and I-A2 autoantibodies in cases of IDDM and NIDDM in southern India. It is concluded that BCG vaccination has an immunomodulatory role in these diseases.**

KEYWORDS: **insulin-dependent diabetes mellitus; BCG vaccination; GAD65 and IA-2 autoantibodies**

Studies on animal models for insulin-dependent diabetes mellitus (IDDM) have shown that injection with BCG vaccine early in age in the diabetes-prone nonobese diabetic (NOD) mouse prevented the development of diabetes.[1] A protective effect was also observed in the spontaneously diabetic BB rat. BCG immunotherapy has also been shown to prevent recurrence of diabetes in islet grafts transplanted into spontaneously diabetic NOD mice.[2] In an open clinical trial in 17 newly diagnosed IDDM patients, intracutaneous administration of 0.1 mL of BCG vaccine led to a clinical remission more frequently than when compared to nontreated controls.[3] There was a debate in *Diabetologia* on the role of BCG vaccination and incidence of diabetes in Swedish children.[4,5] It is still not resolved whether there is a beneficial effect of BCG vaccination on the development of autoimmune diabetes whether given 8 weeks before or 8 weeks after birth.

GAD65 and IA-2 autoantibodies are associated with autoimmune diabetes in the Caucasian population.[6–8] The prevalence of autoantibodies in Indian IDDM patients were similar to that of Caucasians[9] and, in addition, 43% of clinically diagnosed NIDDM patients[10] had either or both autoantibodies, suggesting a high prevalence of slow-onset IDDM in this population.

The aim of the present study was to test whether BCG vaccination is associated with either increased or decreased autoantibody formation in southern Indian diabet-

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TABLE 1. The frequency of autoantibodies in BCG-vaccinated and nonvaccinated diabetic patients

Autoantibody	BCG vaccinated ($n = 86$)	BCG nonvaccinated ($n = 51$)	P value
GAD65 Ab	31 (36%	34 (67%)	<0.0005
ICA512 Ab	16 (19%)	22 (43%)	<0.001

NOTE: The differences in autoantibody frequencies between BCG-vaccinated and nonvaccinated groups were tested using the Chi square test with Yates correction. The probability values were considered significant if lower than 0.05.

TABLE 2. The frequency of autoantibodies in BCG-vaccinated and nonvaccinated type 1 diabetes patients

Autoantibody	BCG vaccinated ($n = 57$)	BCG nonvaccinated ($n = 34$)	P value
GAD65 Ab	31 (54%)	34 (100%)	<0.001
ICA512 Ab	13 (23%)	21 (62%)	<0.001

NOTE: The differences in autoantibody frequencies between BCG-vaccinated and nonvaccinated groups were tested using the Chi square test with Yates correction. The probability values were considered significant if lower than 0.05.

ic patients, which includes both those clinically diagnosed with IDDM and and with NIDDM. The IDDM and NIDDM designation is based on the WHO's clinical classification formulated in 1985. In the revised WHO classification of diabetes mellitus of 1998 based on the etiopathogenestic criteria, all autoatibody-positive diabetic patients are to be diagnosed as having type 1 diabetes mellitus.

In this study we used radioligand binding assay using *in vitro* transcribed and translated ^{35}S-labeled human recombinant GAD65 and IA-2 in patients and controls. The prevalence of GAD65 and IA-2 autoantibodies in healthy controls from southern India are 4% and 2%, respectively. One hundred thirty-seven diabetic patients were assayed for GAD65 and ICA512 autoantibodies. All patients had new-onset or disease of 5 years' or less duration. Of the total number of patients, 86 had been vaccinated with BCG immediately after birth, while 51 did not receive BCG vaccination at all. The BCG vaccination status was ascertained by the identification of the BCG vaccination scars in the left arm at the time of inclusion in the study.

GAD65 antibodies was present in 65 of 137 (47%) and IA-2 antibodies were present in 38 of 137 (28%) patients. The results in TABLE 1 suggest that GAD65 and IA-2 autoantibody frequencies are significantly decreased in BCG-vaccinated diabetic patients compared to those not vaccinated with BCG.

All the autoantibody-positive patients were designated as having "autoimmune diabetes" or type 1 diabetes (T1DM). Ninety-one patients were either GAD65- or IA-2- or both antibody-positive and hence were considered to have T1DM. TABLE 2, in addition, shows the frequency of GAD65 and IA-2 autoantibodies in type 1 diabetes patients. The results show that BCG-vaccinated patients develop significantly

fewer autoantibodies than do those not vaccinated with BCG. It is not clear whether this significant decrease in the autoantibody development in BCG-vaccinated patients is due to switch from Th1 to Th2 response caused by BCG vaccination.

Immunization at birth with vaccines has resulted in complete prevention of diabetes in the NOD mouse and significant reduction in BB rats.[1,2] However, immunization starting after 8 weeks was associated with either increased or no decreased incidence of diabetes.[11] According to J. B. Classen,[12] immunization at birth with BCG in humans was associated with prevention of 50 cases of diabetes per 100,000 immunized, while immunization after 8 weeks with several human vaccines was associated with an increase of 30 cases or more of diabetes per 100,000 immunized.

In conclusion, BCG vaccination has an immunomodulatory role and is associated with decreased autoantibody positivity in south Indian diabetic patients, which is in conformity with the observations from animal models of autoimmune diabetes.

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